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Creating a model antigen system to test the mechanism of GCC-specific tolerance

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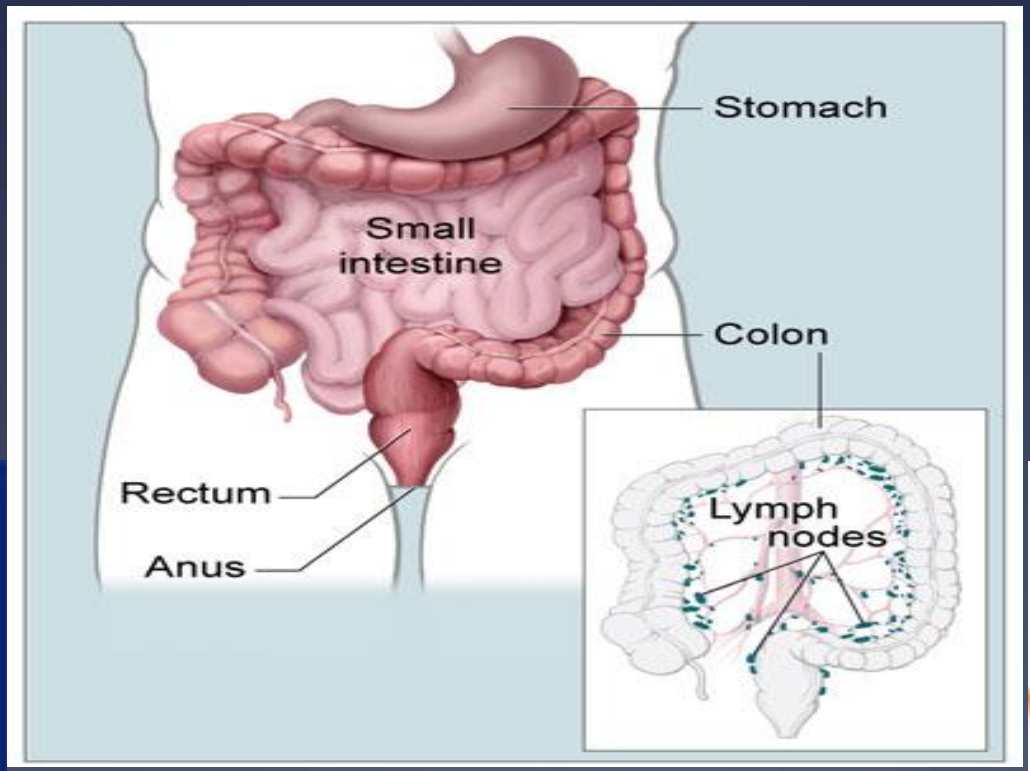
CREATING A MODEL ANTIGEN SYSTEM TO TEST THE MECHANISM OF GCC-SPECIFIC TOLERANCE

- Patrick Ihejirika



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Cancer Cases and Deaths

Leading New Cancer Cases and Deaths – 2012 Estimates

Estimated New Cases*		Estimated Deaths	
Male	**Female**	**Male**	**Female**
Prostate 241,740 (29%)	Breast 226,870 (29%)	Lung & bronchus 87,750 (29%)	Lung & bronchus 72,590 (26%)
Lung & bronchus 116,470 (14%)	Lung & bronchus 109,690 (14%)	Prostate 28,170 (9%)	Breast 39,510 (14%)
Colon & rectum 73,420 (9%)	Colon & rectum 70,040 (9%)	Colon & rectum 26,470 (9%)	Colon & rectum 25,220 (9%)
Urinary bladder 55,600 (7%)	Uterine corpus 47,130 (6%)	Pancreas 18,850 (6%)	Pancreas 18,540 (7%)
Melanoma of the skin 44,250 (5%)	Thyroid 43,210 (5%)	Liver & intrahepatic bile duct 13,980 (5%)	Ovary 15,500 (6%)
Kidney & renal pelvis 40,250 (5%)	Melanoma of the skin 32,000 (4%)	Leukemia 13,500 (4%)	Leukemia 10,040 (4%)
Non-Hodgkin lymphoma 38,160 (4%)	Non-Hodgkin lymphoma 31,970 (4%)	Esophagus 12,040 (4%)	Non-Hodgkin lymphoma 8,620 (3%)
Oral cavity & pharynx 28,540 (3%)	Kidney & renal pelvis 24,520 (3%)	Urinary bladder 10,510 (3%)	Uterine corpus 8,010 (3%)
Leukemia 26,830 (3%)	Ovary 22,280 (3%)	Non-Hodgkin lymphoma 10.320 (3%)	Liver & intrahepatic bile duct 6,570 (2%)
Pancreas 22,090 (3%)	Pancreas 21,830 (3%)	Kidney & renal pelvis 8,650 (3%)	Brain & other nervous system 5,980 (2%)
All sites 848,170 (100%)	All sites 790,740 (100%)	All sites 301,820 (100%)	All sites 275,370 (100%)

*Excludes basal and squamous cell skin cancers and in situ carcinoma except urinary bladder.

Colorectal Cancer

- The main treatment options include:
  Surgery, chemo-, radiation, biological therapy.
  All treatments present a risk of side effects

- Current standard of care: FOLFOX, FOLFIRI, etc
  - ✓ *Developed in 1980*
  - ✓ *Minimal response rate*
  - ✓ *Prognosis: 5 year survival rate of 63%*

Colorectal Cancer Immunotherapy

Meta-analysis of clinical trials reveal a very weak clinical response rate (<1%) for active specific immunotherapy procedures currently available for colorectal cancer

Nagorsen D, et al. Clin Cancer Res 2006;12:3064-9.

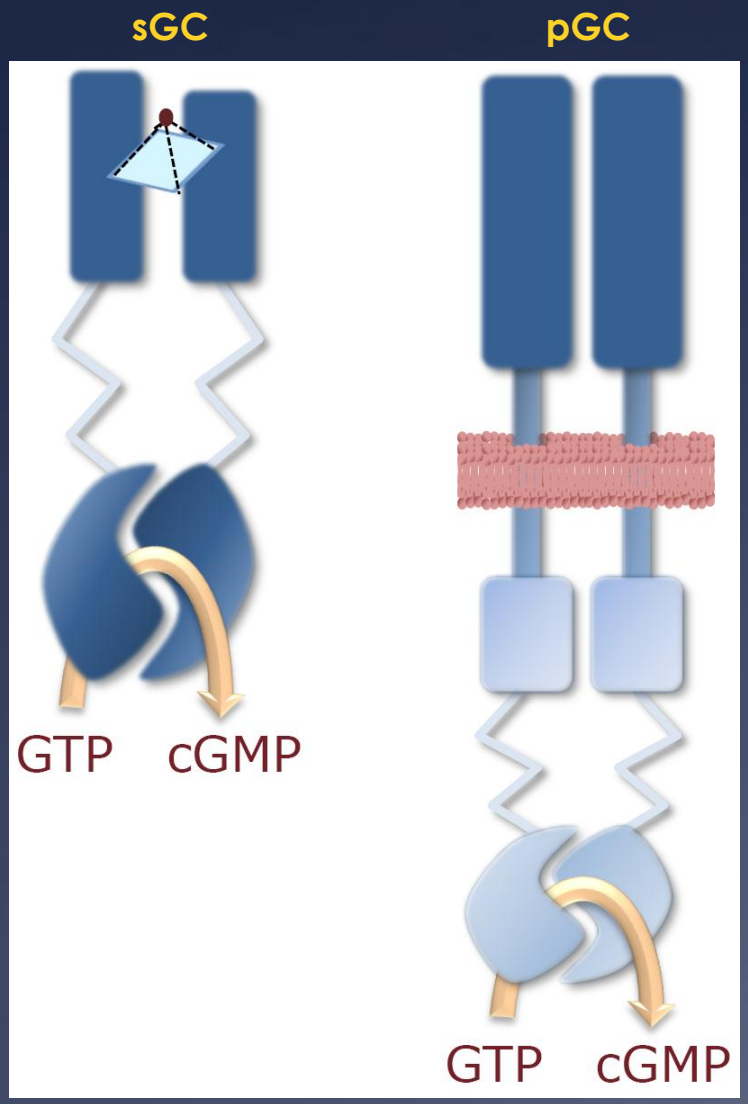
Failure is due to the lack of molecularly defined tumor-associated antigens that can be reliably considered:

- ***Tumor-specific***
- ***Sufficiently immunogenic***
- ***Shared among different patients***

Adapted from: Dalerba P, et al. Crit Rev Oncol Hemat 2003;46(1):33-57.

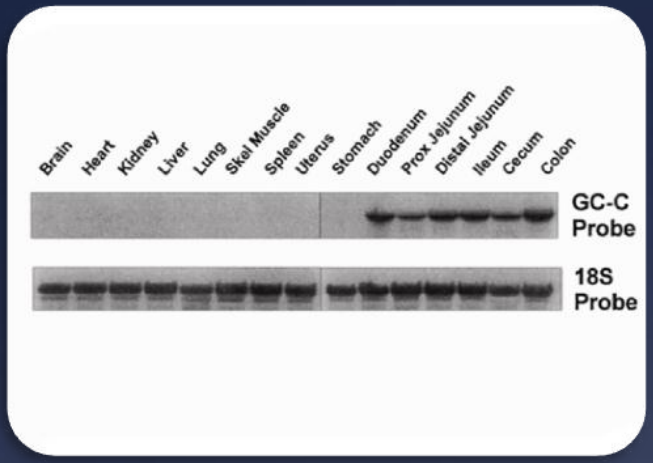
Colorectal Cancer TAA		
Mutant Self Proteins		
K-ras	p53	
Oncofetal / Cancer Testis Antigens		
βhCG	Gastrin	5T4
Overexpressed Self Antigens		
p53	MUC1	SART
Sialyl-Tn	Her2/neu	ART
Survivin	CD55	Ep-CAM
Carcinoembryonic Antigen (CEA)		
Tissue-Specific Differentiation Antigens		
	?	

Guanylyl Cyclases

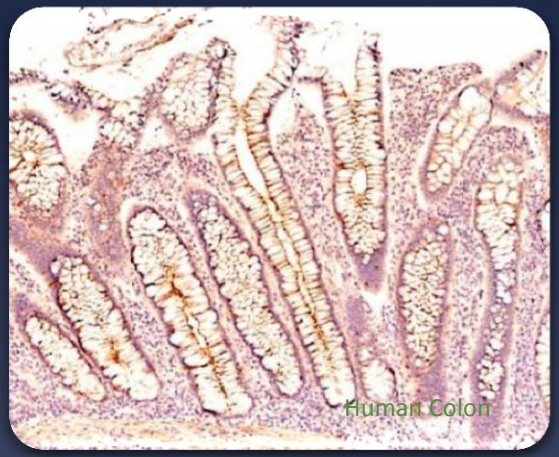


Guanylyl Cyclases		
	Isoforms	Ligands
Natriuretic Peptide Receptors	GCA	ANP, BNP
	GCB	CNP
Intestinal Peptide Receptor	GCC	ST Guanylin Uroguanylin
Orphan Receptors	GCD GCE GCF GCG	
Soluble	sGC	NO

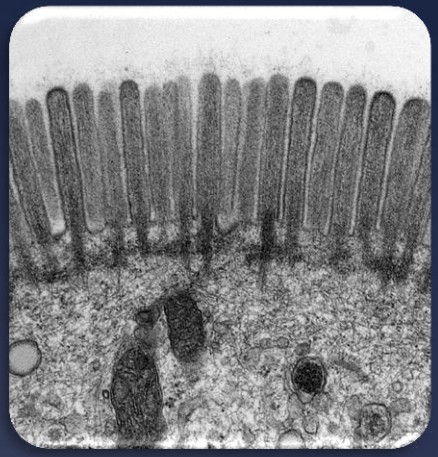
Mucosa-Restricted GCC Expression



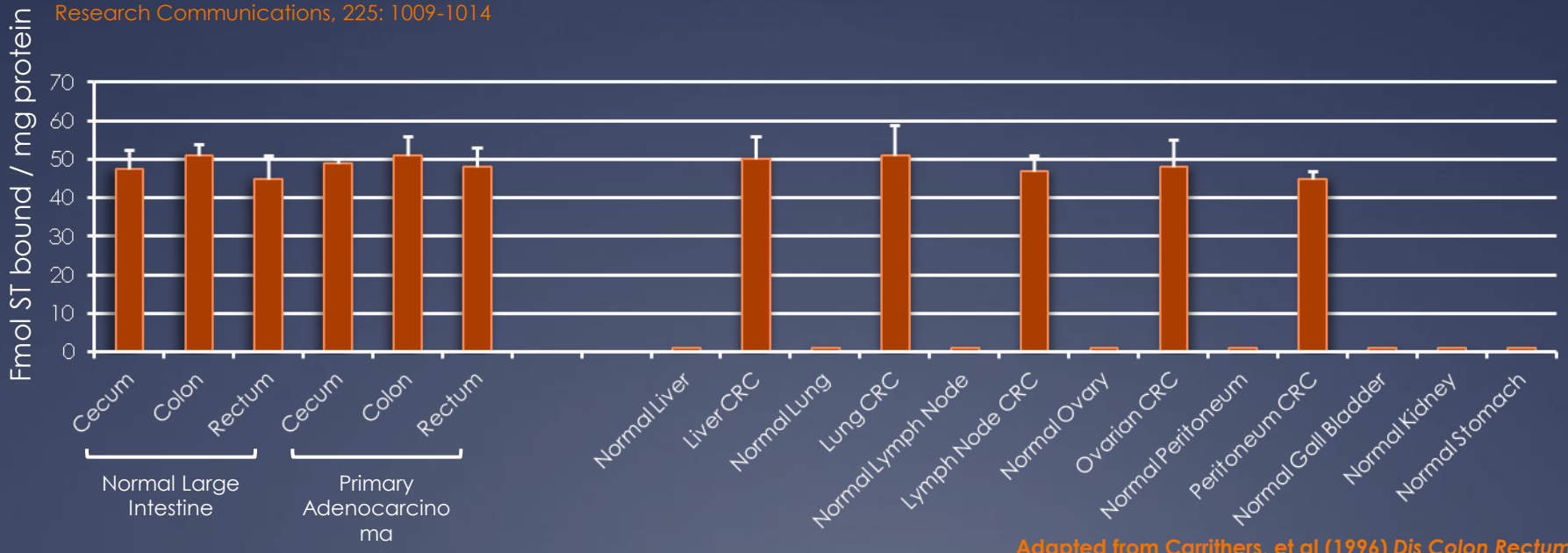
Swensen et al. 1996. Biochemical and Biophysical Research Communications, 225: 1009-1014



S Schulz et al, unpublished data

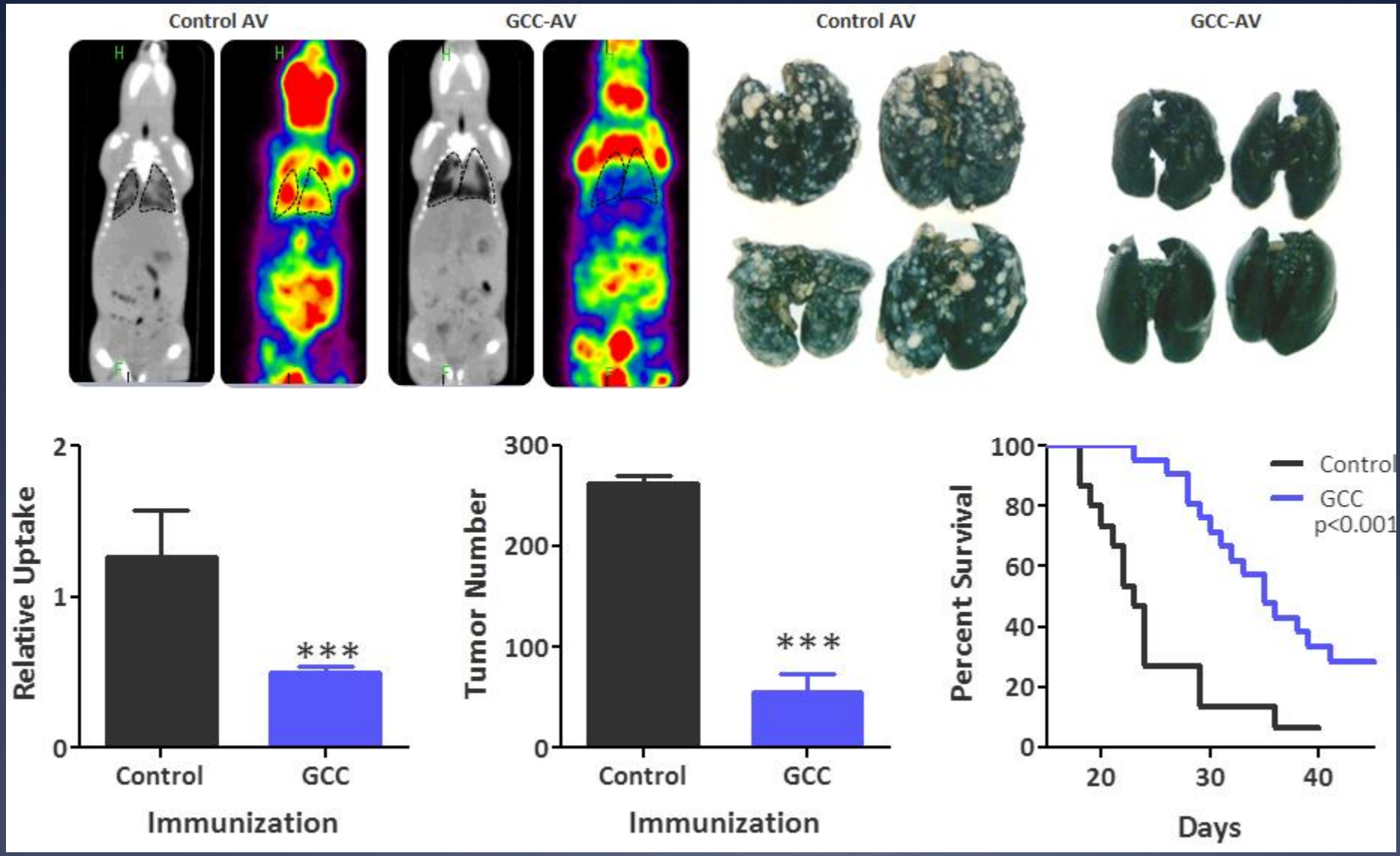


E Lin et al, unpublished data

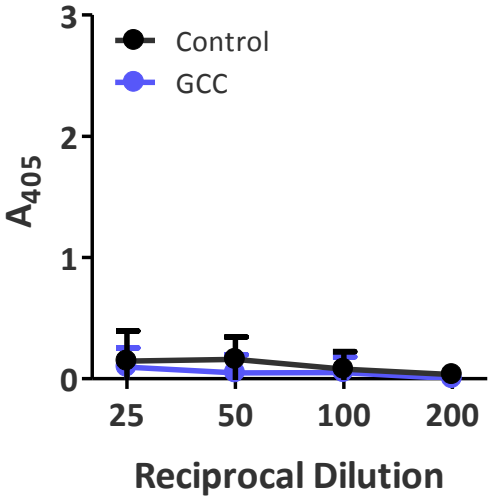


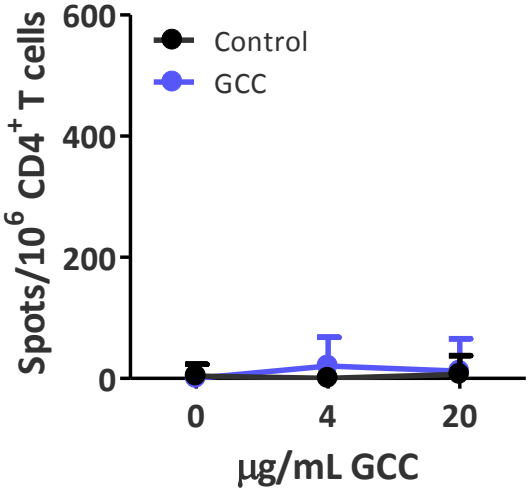
Adapted from Carrithers, et al (1996) *Dis Colon Rectum*

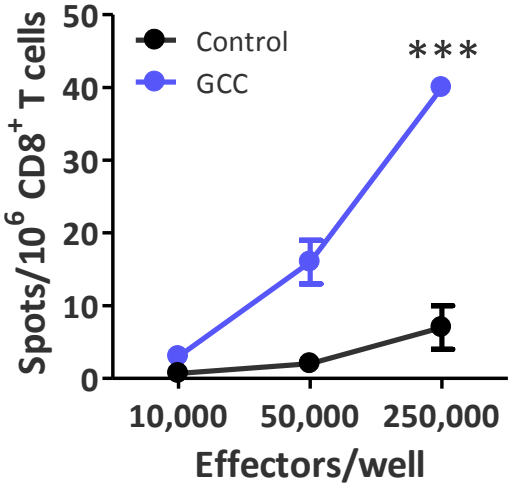
Anti-Tumor Immunity



GCC-Specific Immune Responses

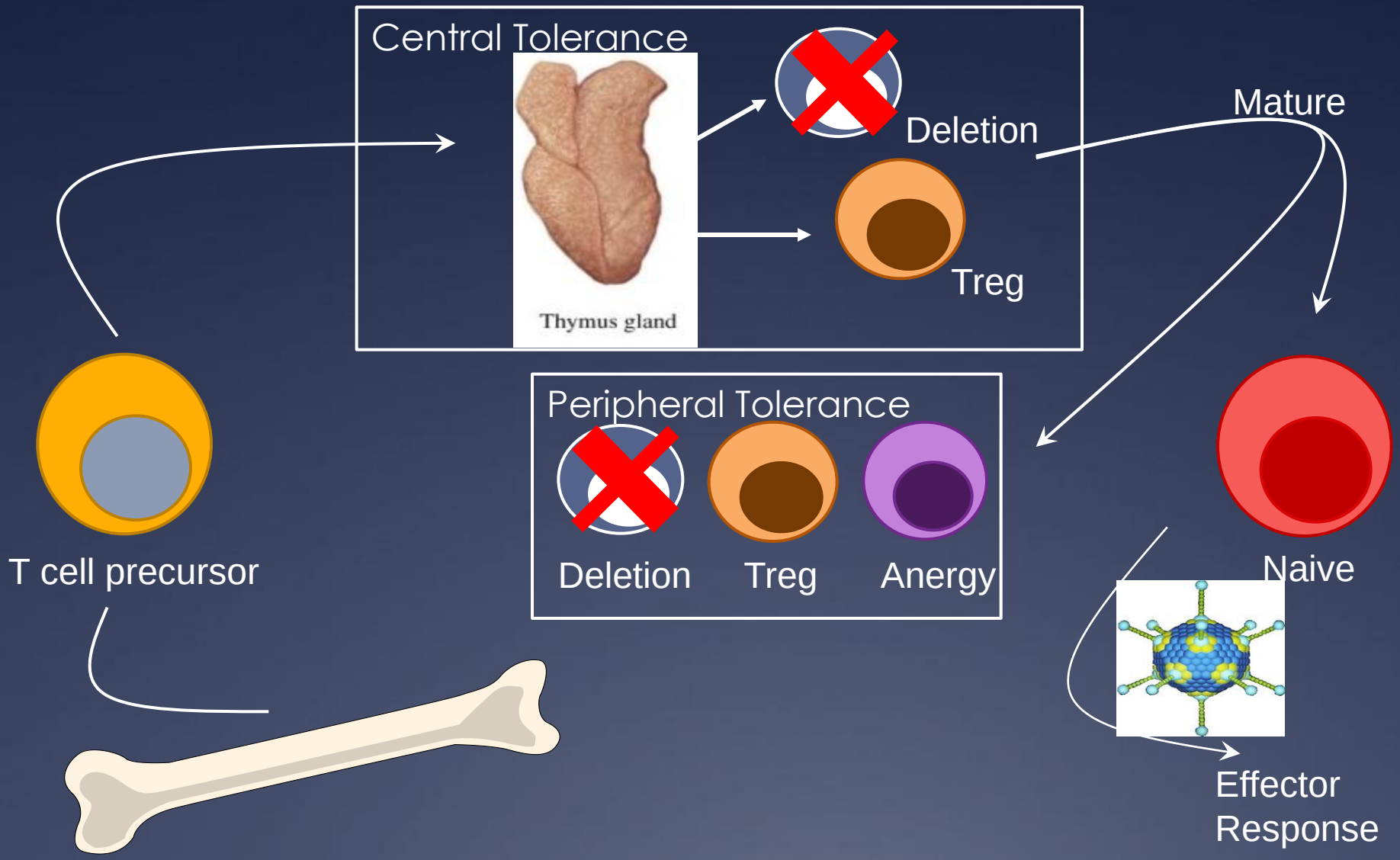




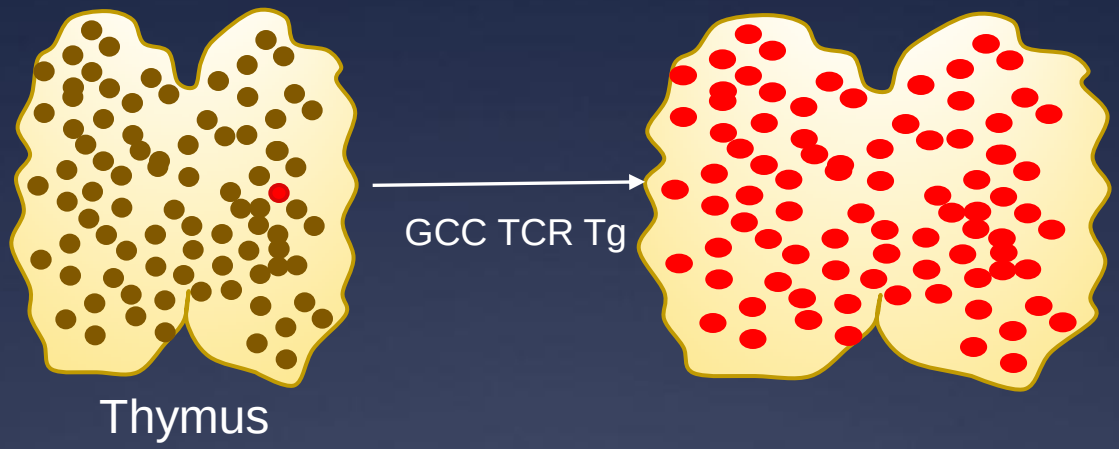


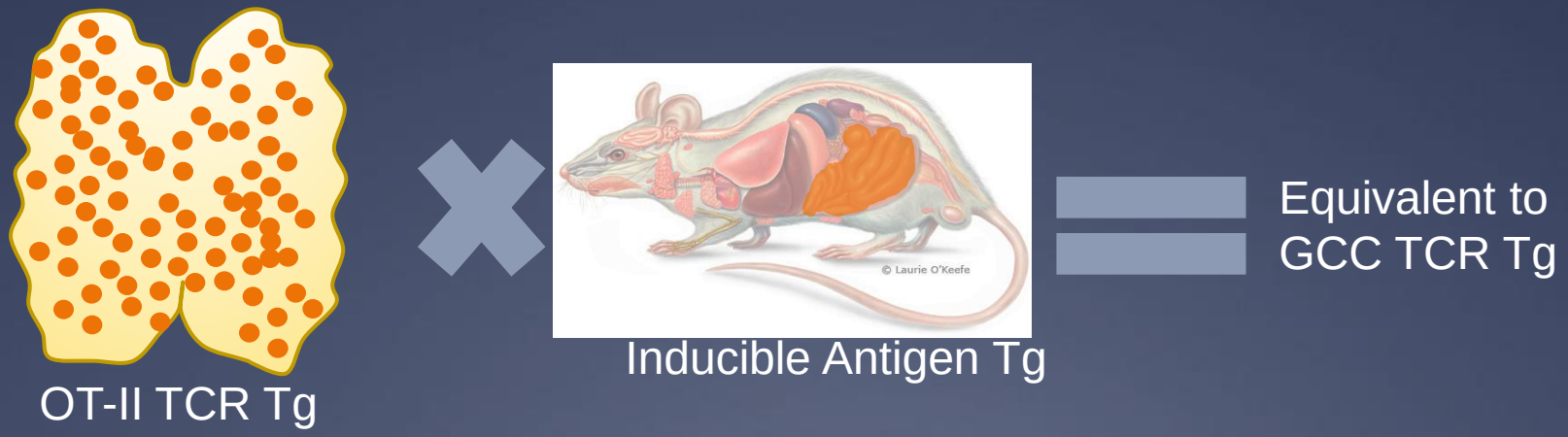
A. Snook et al. JNCI 2008; 100(13):950-96

Predicted CD4 Tolerance Mechanism

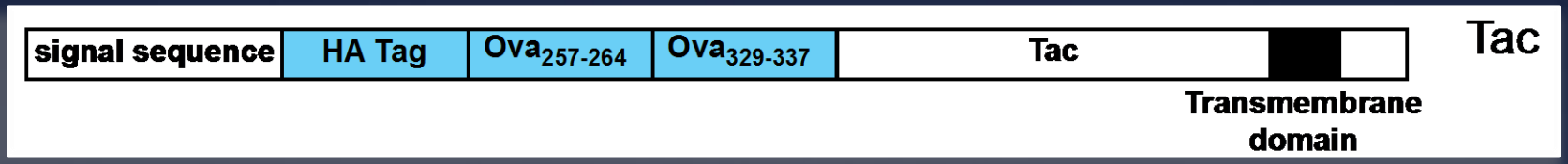


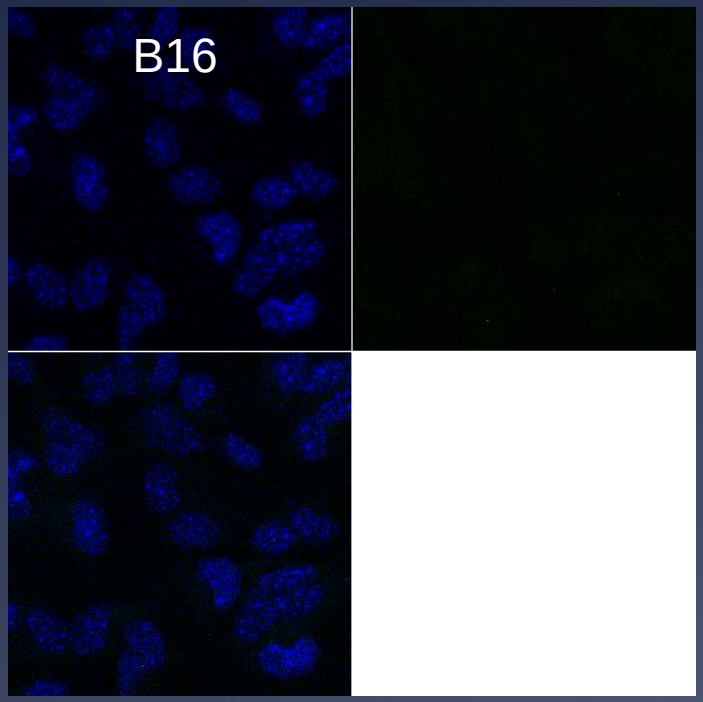
Model System

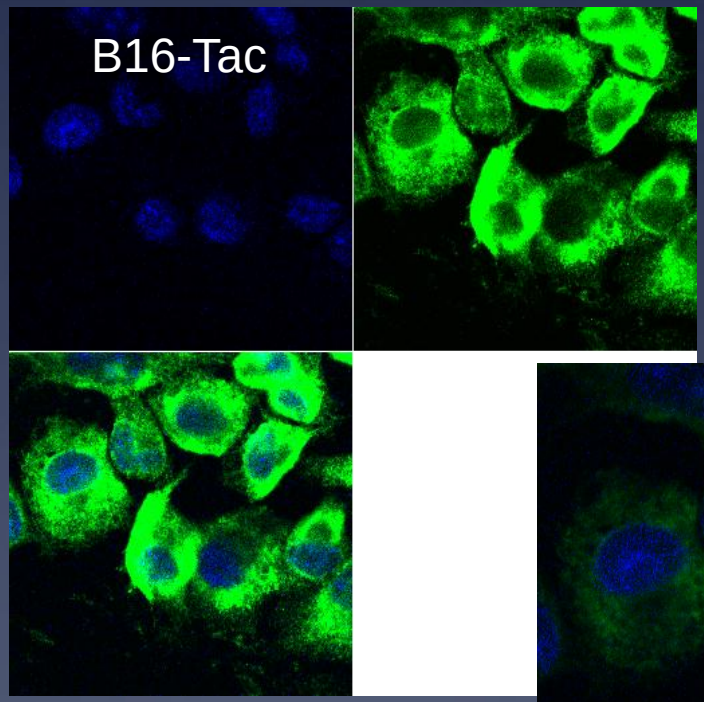




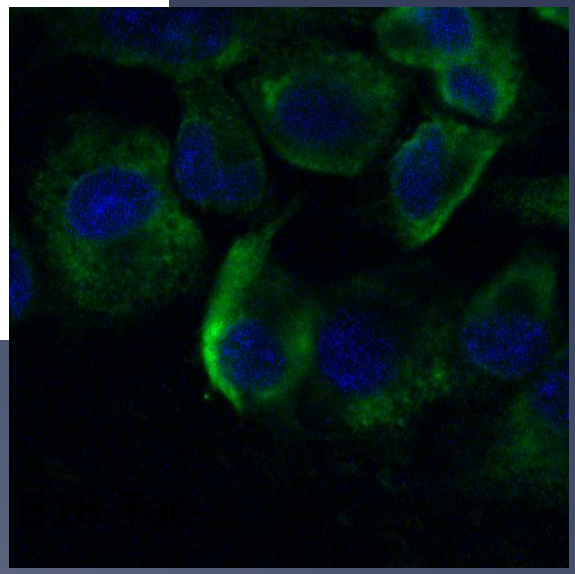
Tac







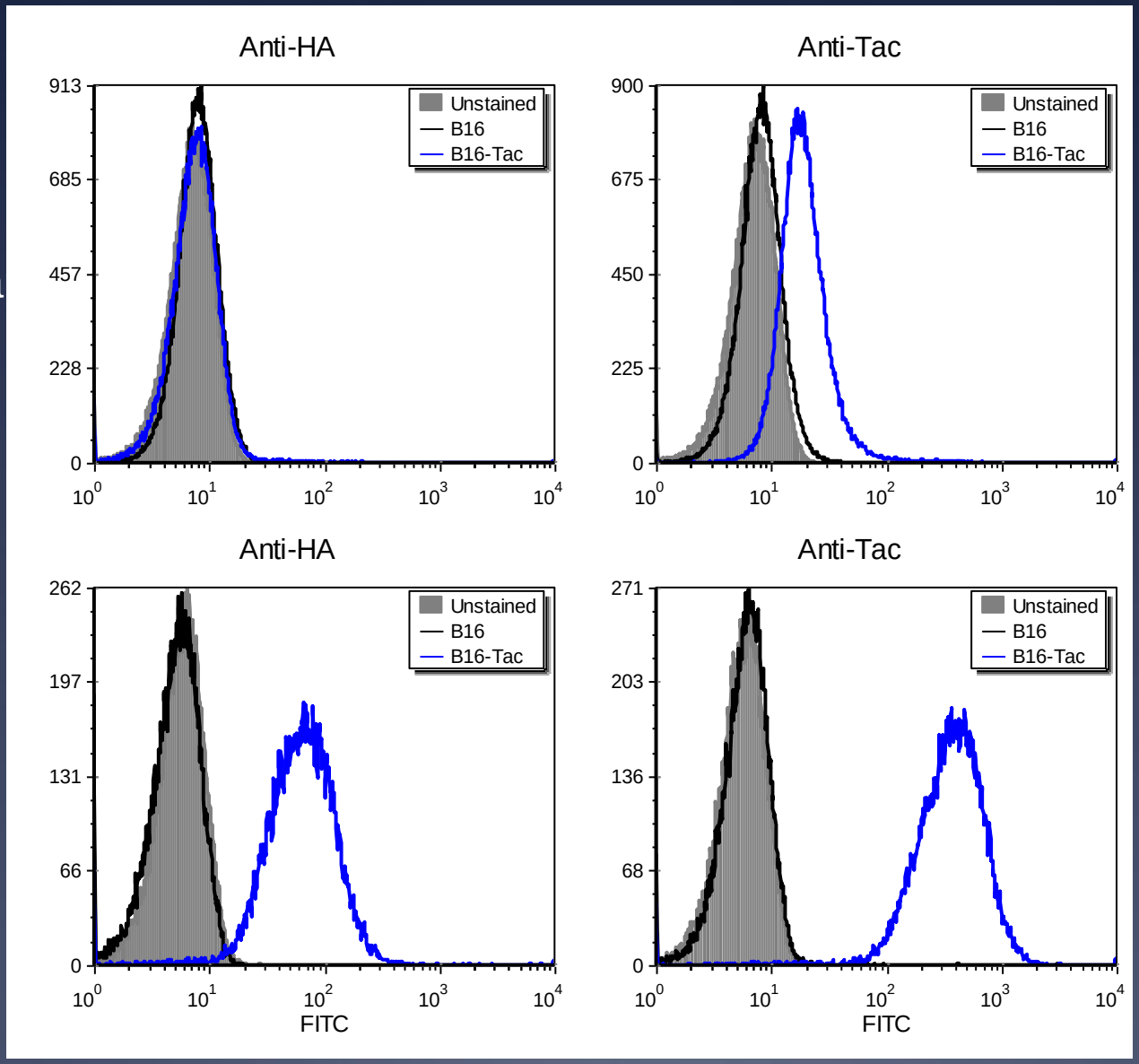
6μm slice



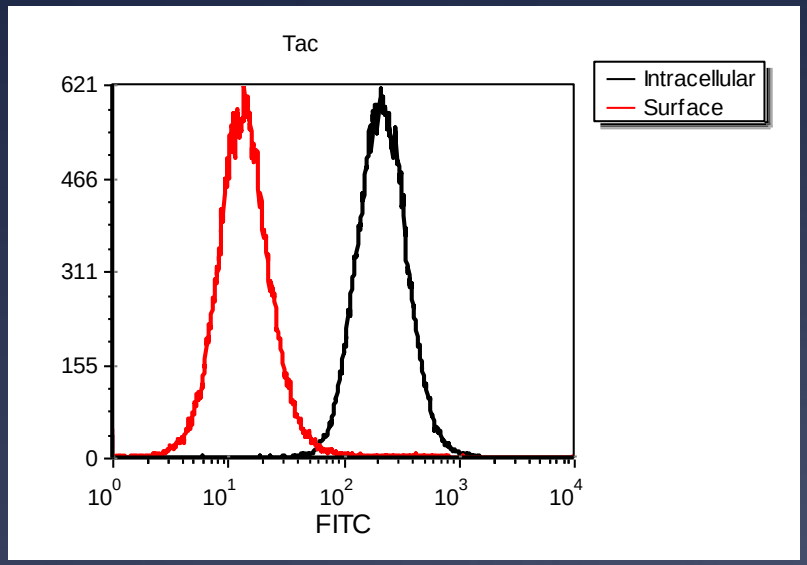
Tac FACS

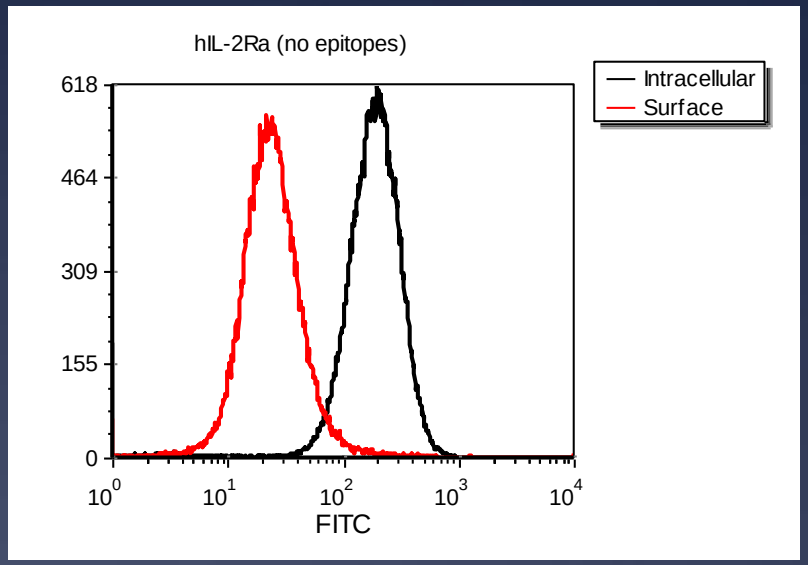
Extracellula

Intracellular



Epitopes and surface expression



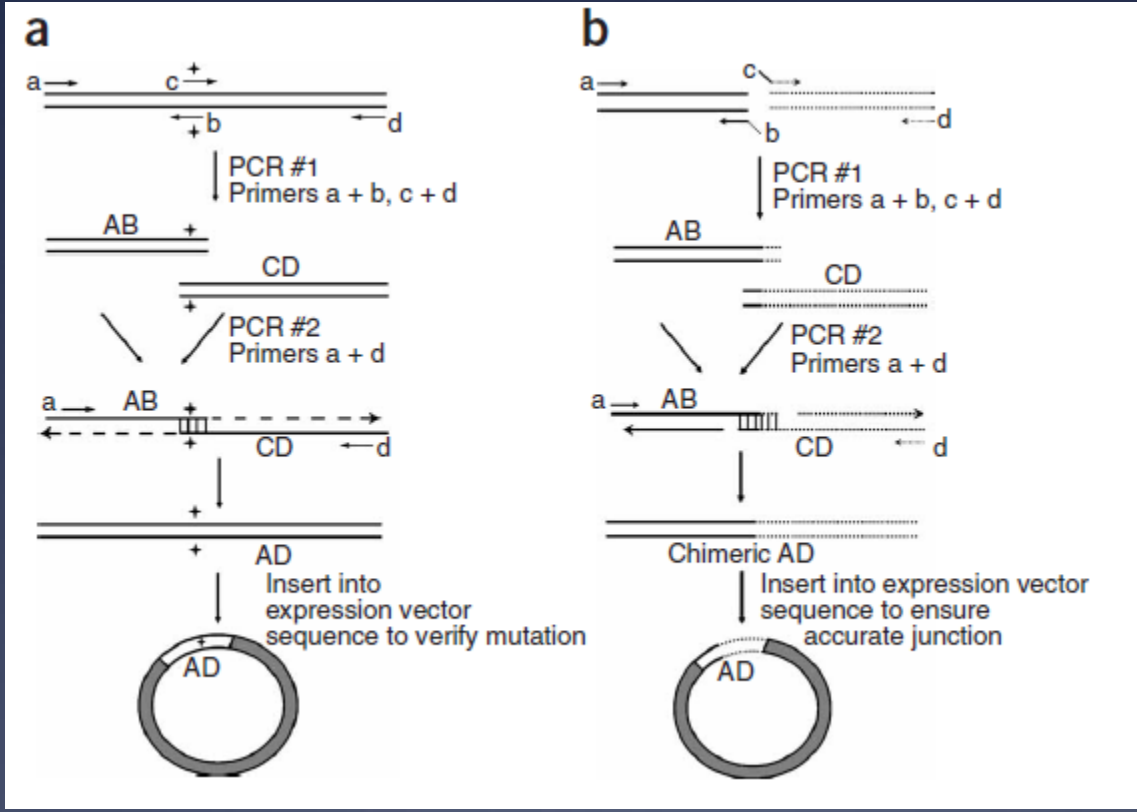


Creating GCC-based model antigen

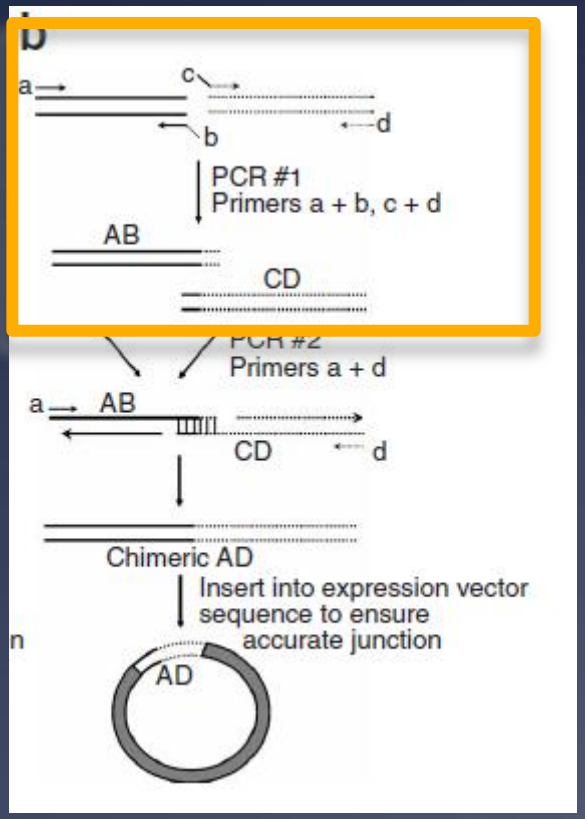
Steps:

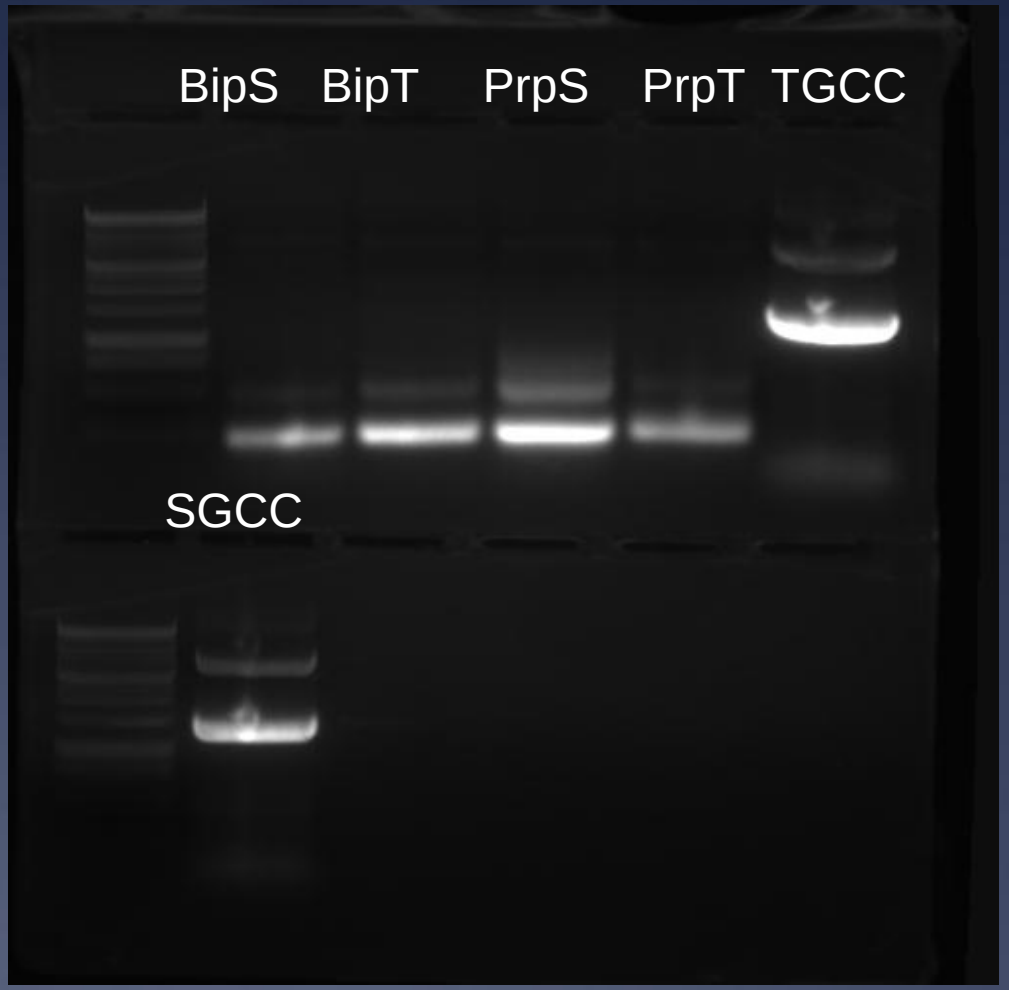
- cloning different epitopes and signal sequences into GCC:
  - ✓ Bip and Prp
- clone the constructs into mammalian expression plasmid (retrovirus)
- create cell lines expressing the constructs by infecting them with retrovirus containing our constructs
- test the new cell lines for 1) Chimeric antigen expression, 2) Chimeric antigen subcellular location, 3) epitope presentation
- work with Dr Eisenlohr's lab to see how different signal sequences and model epitopes affect our new model antigen.

Overhang PCR

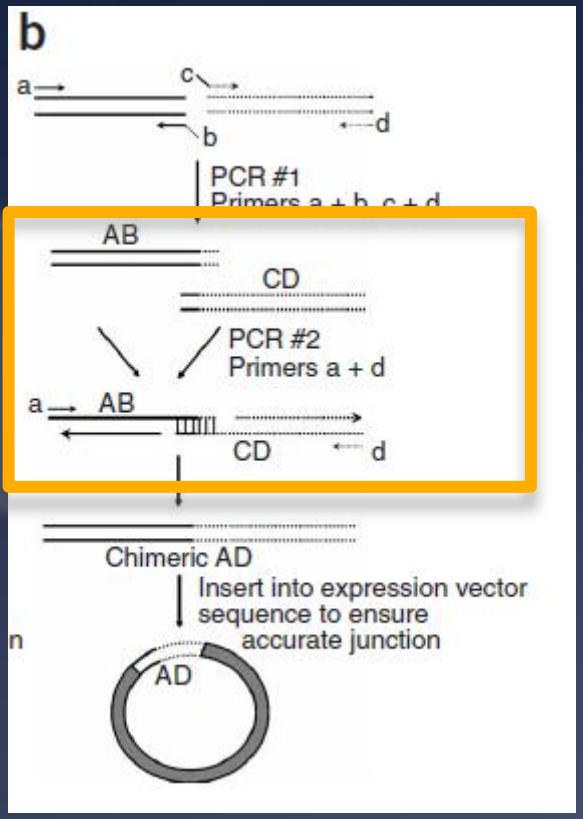


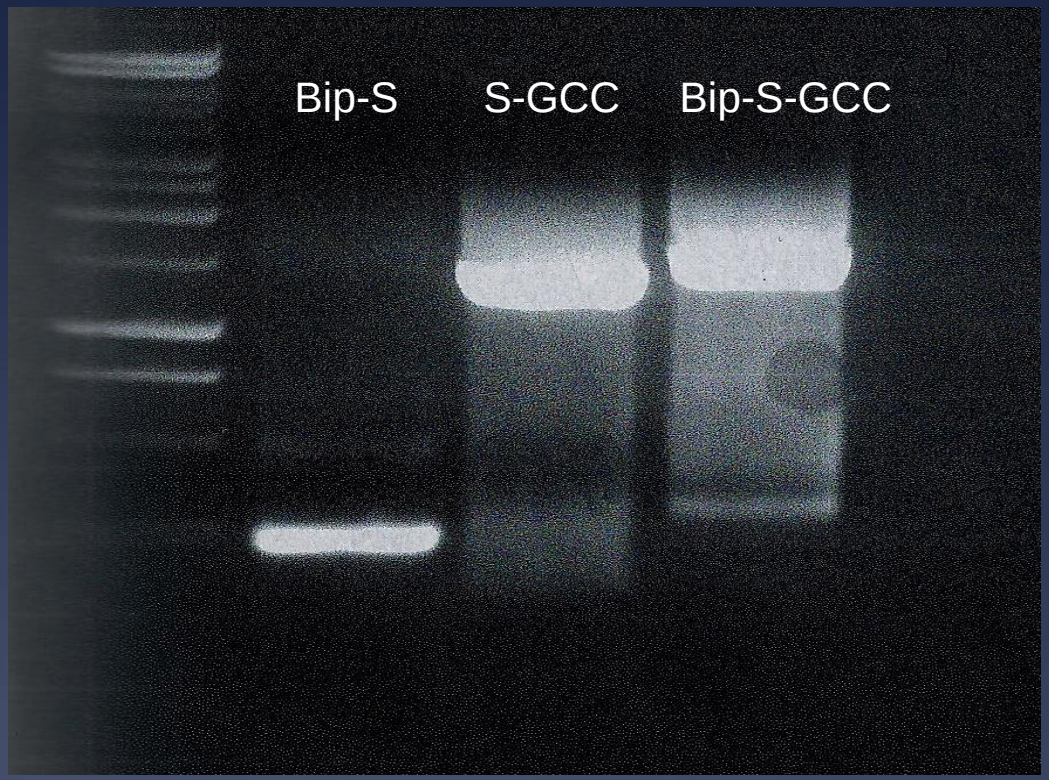
PCR Epitopes and GCC



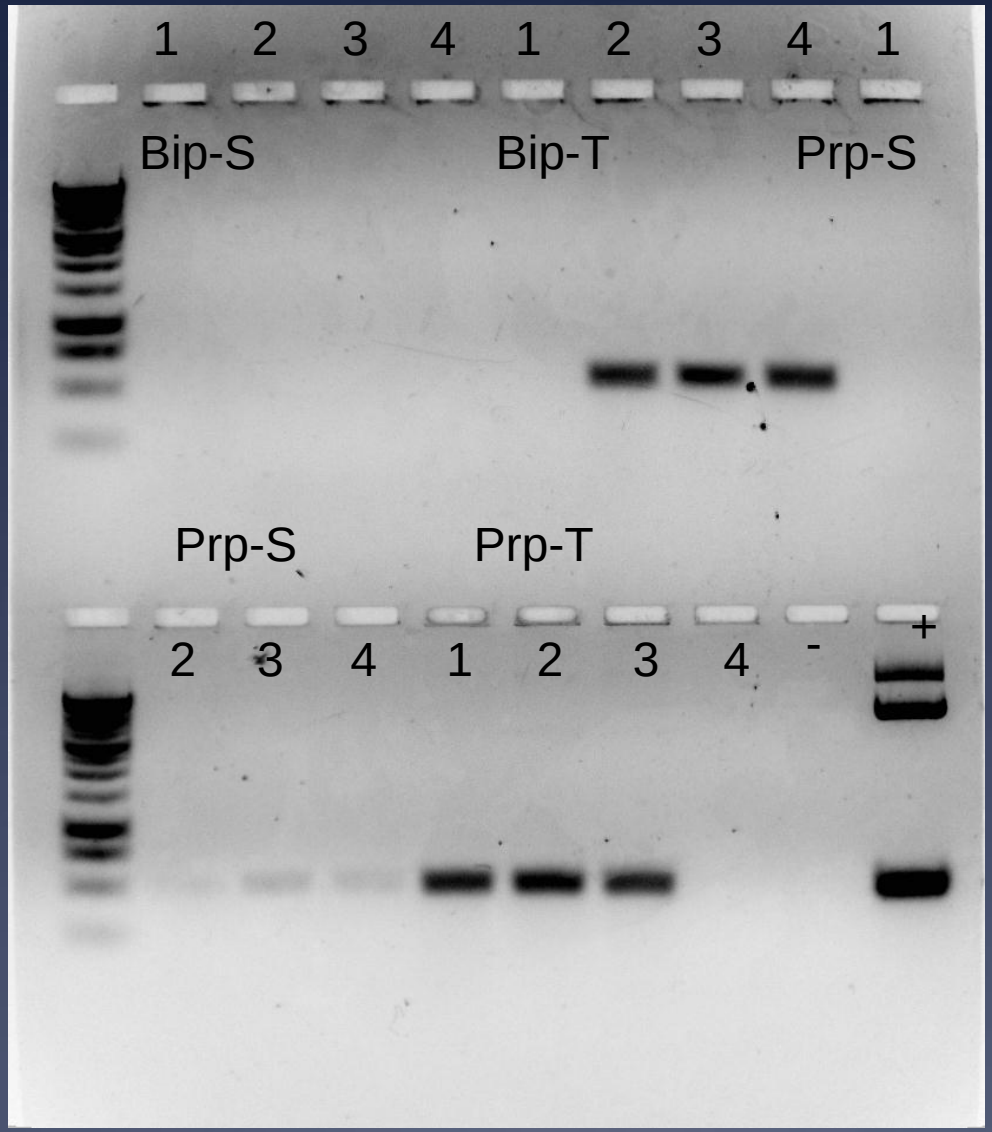


Overhang PCR





Screening pENTR clones



Cutting inserts out

- Sequenced pENTR clones
- Cut inserts out with NotI and SalI
- Cut pMSCV-Puro with NotI and SalI
- Ligated inserts with pMSCV-Puro
- Transformed bacteria
- Screened colonies

Results

Summary/Future Goal

- Create stable cell lines expressing the constructs by target cell lines with retrovirus containing our constructs.
- Test the new cell lines for 1) chimeric antigen expression, 2) define the subcellular location or our antigen, 3) quantify epitope presentation
- Work with Dr. Eisenlohr's lab to see how different signal sequences and model epitopes affect our new model antigen.

Thank You!

- Thomas Jefferson University:
- Elizabeth Rappaport MD
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